



Modeling and simulation of gene regulatory networks 4

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INRIA Grenoble - Rhône-Alpes and IBIS



- IBIS: systems biology group at INRIA/Université Joseph Fourier/CNRS
 - Analysis of bacterial regulatory networks by means of models and experiments
 - Biologists, computer scientists, mathematicians, physicists, ...

<http://ibis.inrialpes.fr>

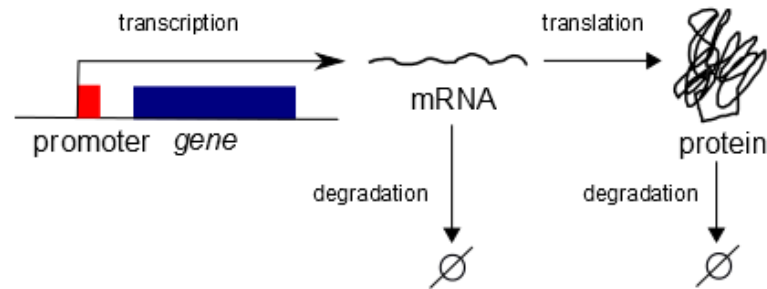


Overview

1. Gene regulatory networks in bacteria
2. Deterministic modeling of gene regulatory networks
3. Qualitative modeling of gene regulatory networks
- 4. Stochastic modeling of gene regulatory networks**
5. Some current issues and perspectives

Gene expression

- Typically, and simplifying quite a bit, **gene expression** in bacteria involves:
 - Transcription by RNAP (mRNA)
 - Translation by ribosomes (proteins)
 - Degradation of mRNA and protein

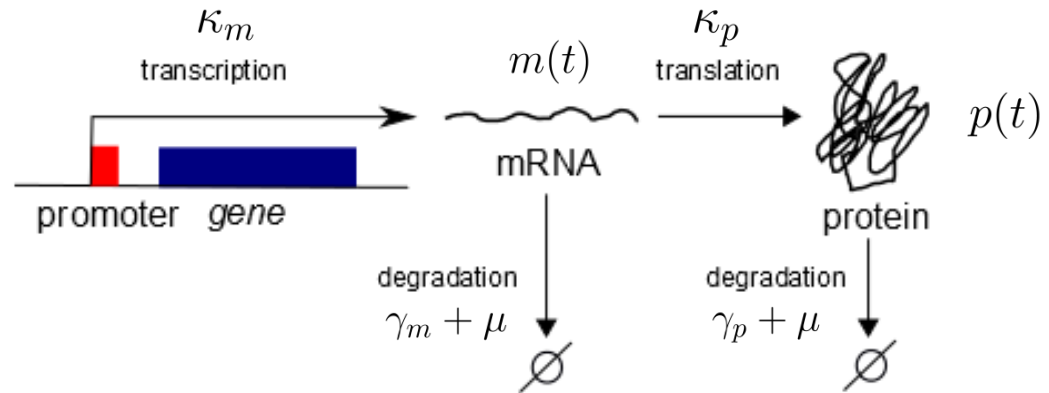


Modeling of gene regulatory networks

- ODE model of gene expression, distinguishing **transcription** and **translation**

$$\dot{m} = \kappa_m - (\gamma_m + \mu) m$$

$$\dot{p} = \kappa_p m - (\gamma_p + \mu) p$$



$m(t) \geq 0$, concentration mRNA

$p(t) \geq 0$, concentration protein

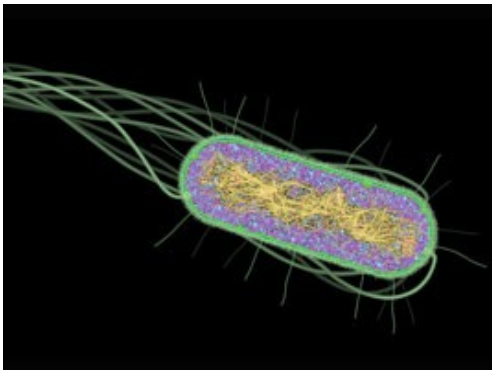
$\kappa_m, \kappa_p > 0$, synthesis rate constants

$\gamma_m, \gamma_p > 0$, degradation rate constants

$\mu \geq 0$, growth rate

Stochasticity in gene expression

- ODE models make abstraction of underlying biochemical reaction processes involved in gene expression that may not be warranted
Kaern et al. (2005), Nat. Rev. Genet., 6(6):451-464
- Gene expression is **stochastic** instead of **deterministic** process
 - Underlying biochemical reactions are stochastic processes
 - Probability of reaction to occur depends on random encounters of molecules in cell



Goodsell (2010), *The Machinery of Life*, Springer, 2nd ed.

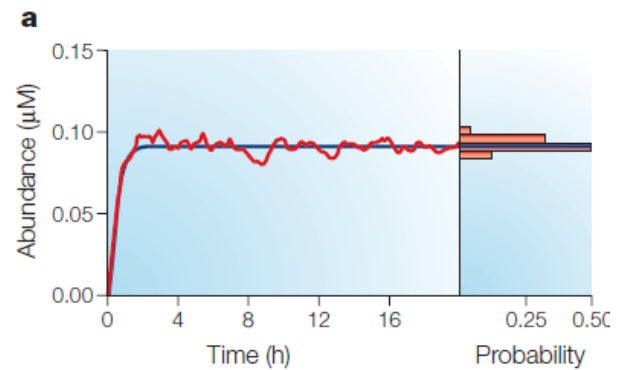
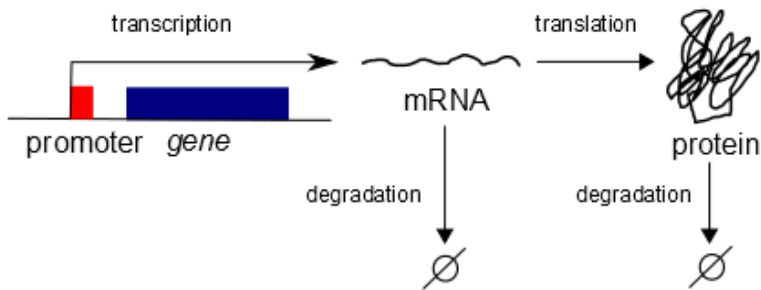
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- Gene expression is **stochastic** instead of **deterministic** process
 - Underlying biochemical reactions are stochastic processes
 - Probability of reaction to occur depends on random encounters of molecules in cell
- **Discrete** number of molecules of reaction species, instead of **continuous** concentrations

Some reactions species involved in gene expression have very low copy numbers (1-10)

Stochasticity in gene expression

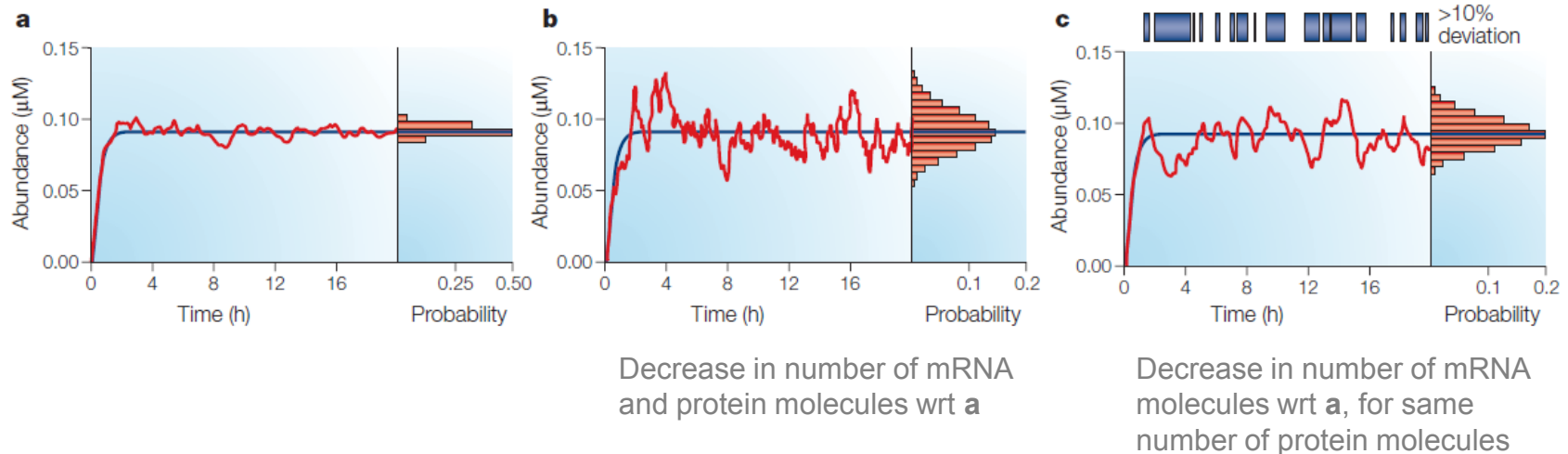
- Stochasticity in gene expression leads to **noise**
Fluctuations in mRNA and protein concentrations



Kaern *et al.* (2005), *Nat. Rev. Genet.*, 6(6):451-464

Stochasticity in gene expression

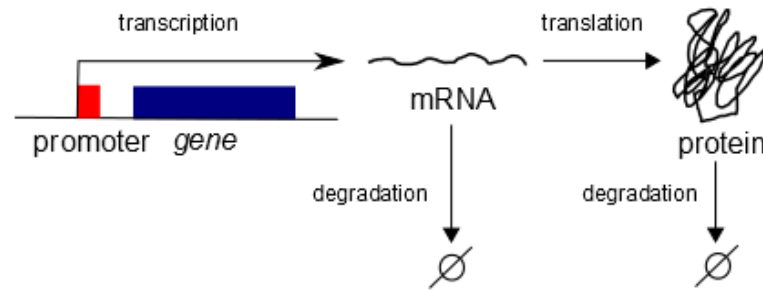
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Fluctuations in mRNA and protein concentrations
- Noise amplified by small number of molecules



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Stochasticity in gene expression

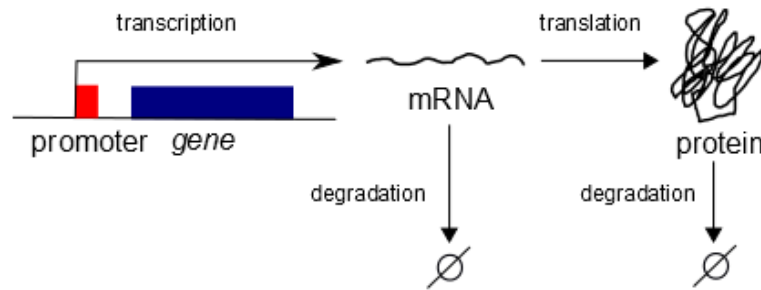
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- Different types of noise:
 - **Intrinsic noise:** fluctuations due to stochasticity of processes involved in gene expression (transcription, translation, ...)

Stochasticity in gene expression

- Stochasticity in gene expression leads to **noise**
Fluctuations in mRNA and protein concentrations
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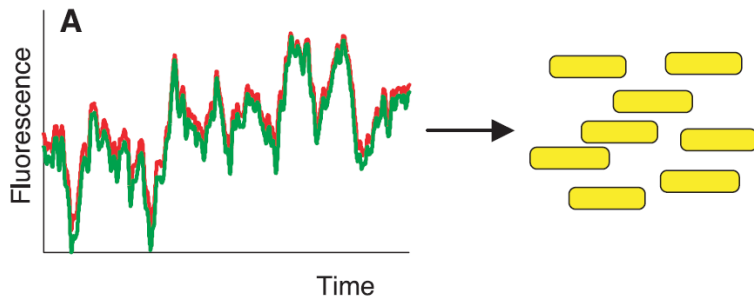


- Different types of noise:
 - **Intrinsic noise:** fluctuations due to stochasticity of processes involved in gene expression (transcription, translation, ...)
 - **Extrinsic noise:** fluctuations due to variability in external factors (temperature, ribosome availability, ...). Impact on rate constants.

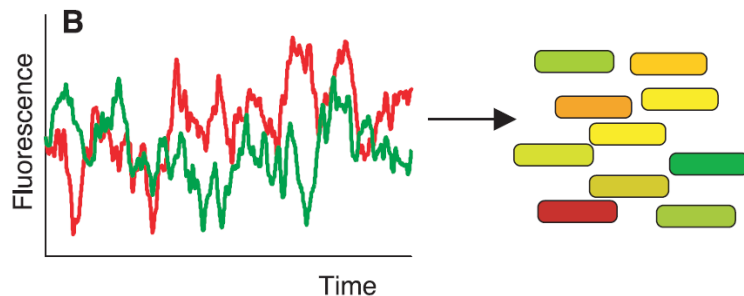
Stochasticity in gene expression

- Experimental discrimination between intrinsic and extrinsic noise

Expression in a single cell with two different reporter genes (*gfp* and *cfp*) controlled by same promoter



No intrinsic noise, so relative amount of both proteins is constant over time and across individual cells in population



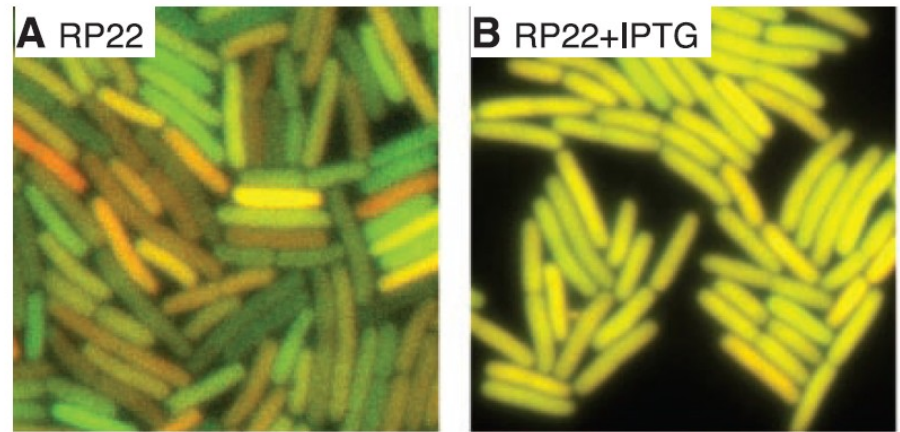
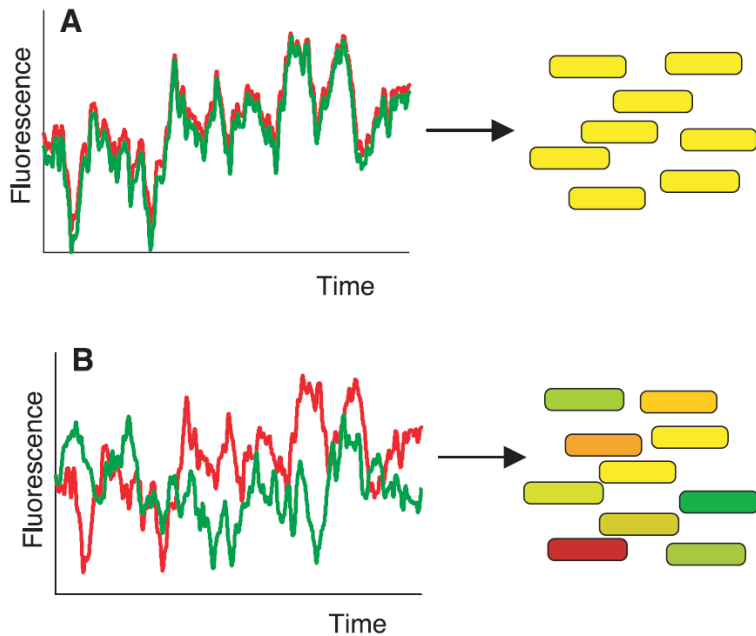
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Elowitz *et al.* (2002), *Science*, 297(5584):1183-6

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Expression in a single cell with two different reporter genes controlled by same promoter

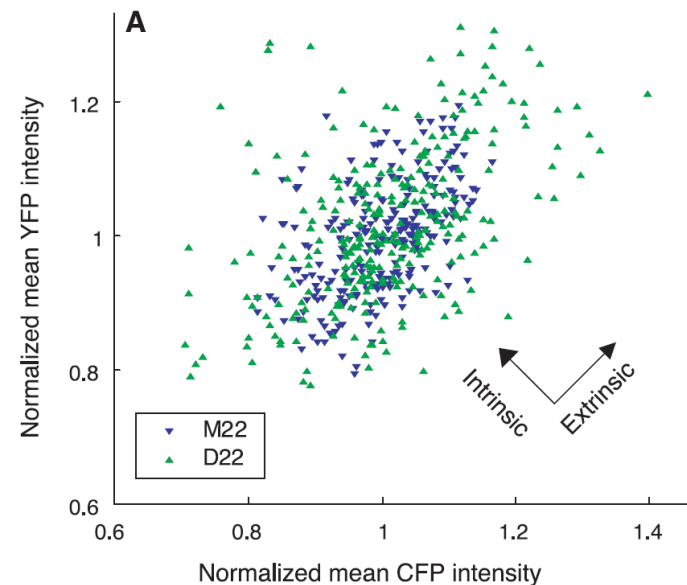
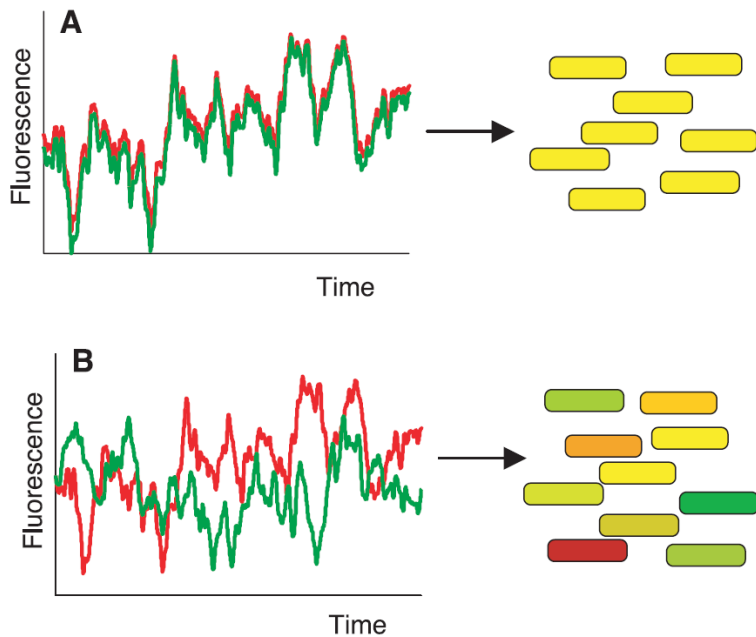


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Stochasticity in gene expression

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Expression in a single cell with two different reporter genes controlled by same promoter



Elowitz *et al.* (2002), *Science*, 297(5584):1183-6

Stochasticity in gene expression

- Major question is how cells both **tolerate and exploit** noise.

Rao *et al.* (2002), *Nature*, 420(6912):231-237

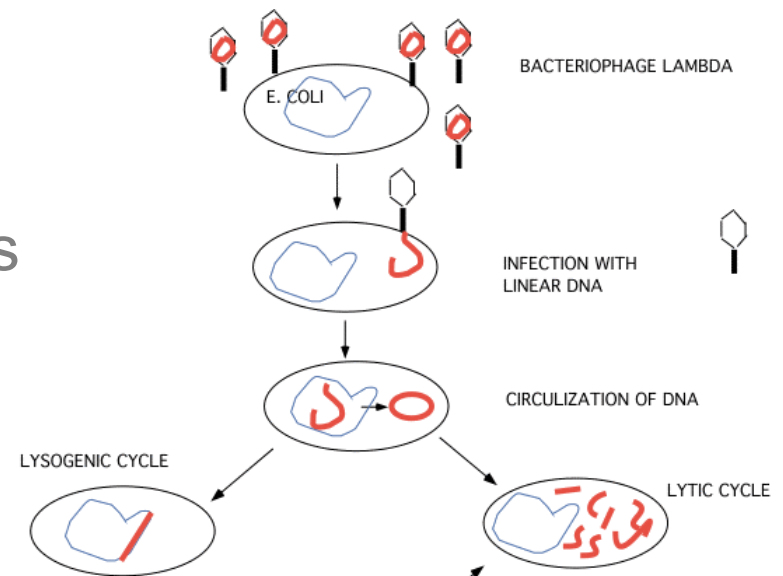
Raj and van Oudenaarden (2008), *Cell*, 135(2):216-26

- Most cellular processes are **robust** to noise, despite stochasticity of underlying system of biochemical reactions

- Sometimes, intracellular noise drives **population heterogeneity** that may be beneficial for a species

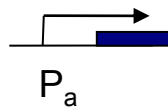
After infection, only fraction of cells lyse

- ODE models are not suitable for studying origin and effects of noise



Stochastic models

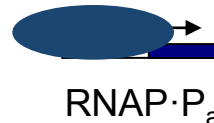
- **Stochastic models** of gene regulation are more appropriate
- Number of molecules of each species i at time-point t represented by discrete variable $X_i(t) \in \mathbf{N}$



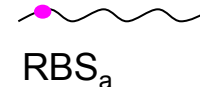
X_1



X_2



X_3

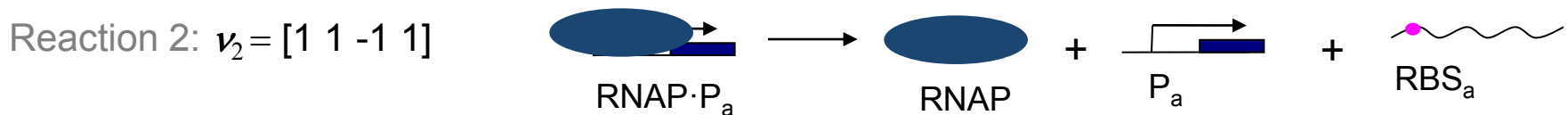
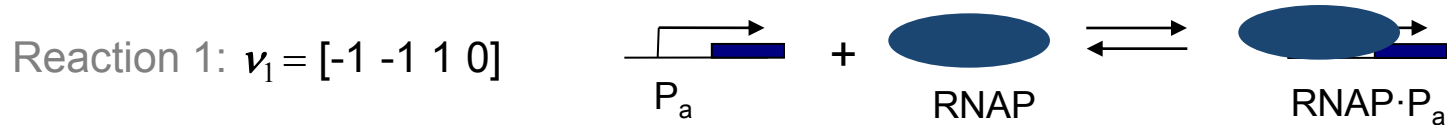


X_4

Stochastic models

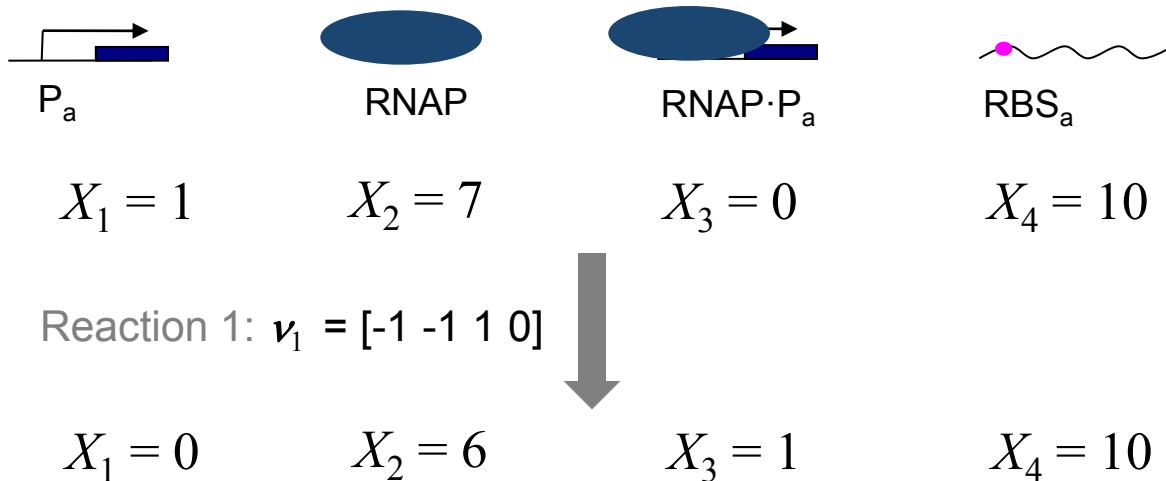
- **Stochastic models** of gene regulation are more appropriate
- Number of molecules of each species i at time-point t represented by discrete variable $X_i(t) \in \mathbb{N}$
- Reactions between molecular species lead to change in state of system from $\mathbf{X}(t)$ to $\mathbf{X}(t+\Delta t)$ over time-interval Δt , where $\mathbf{X} = [X_1, \dots, X_n]'$

Change of state by reaction k described by vector \mathbf{v}_k



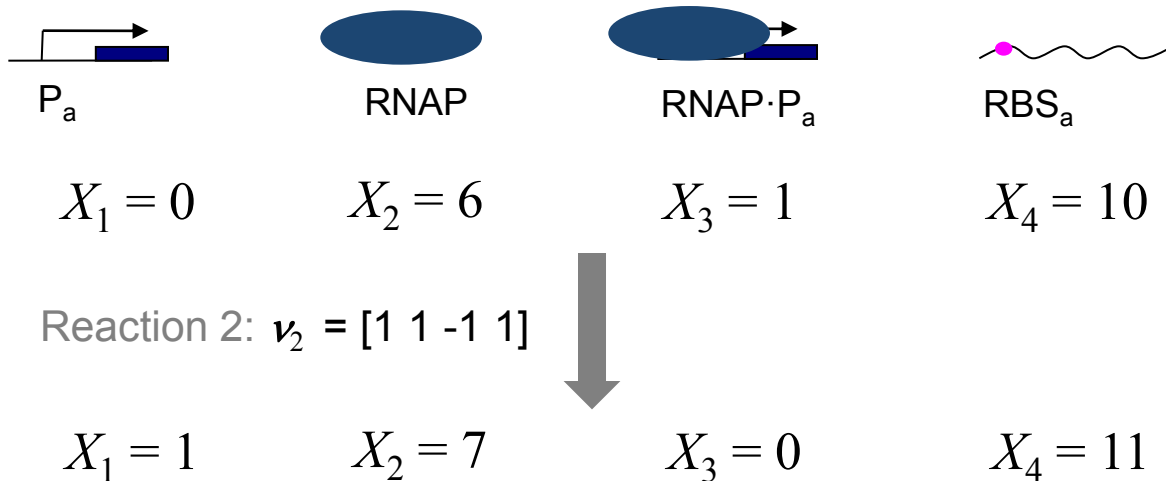
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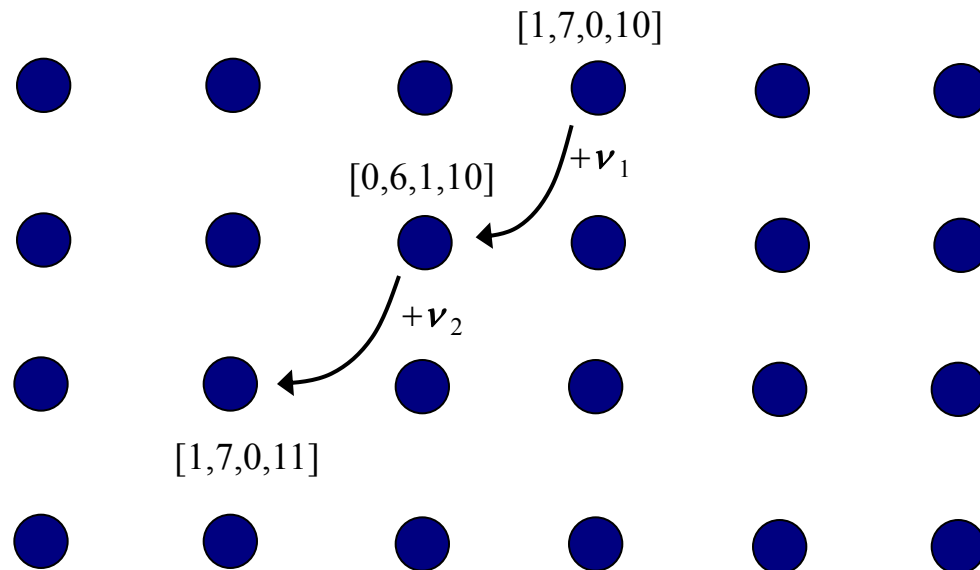
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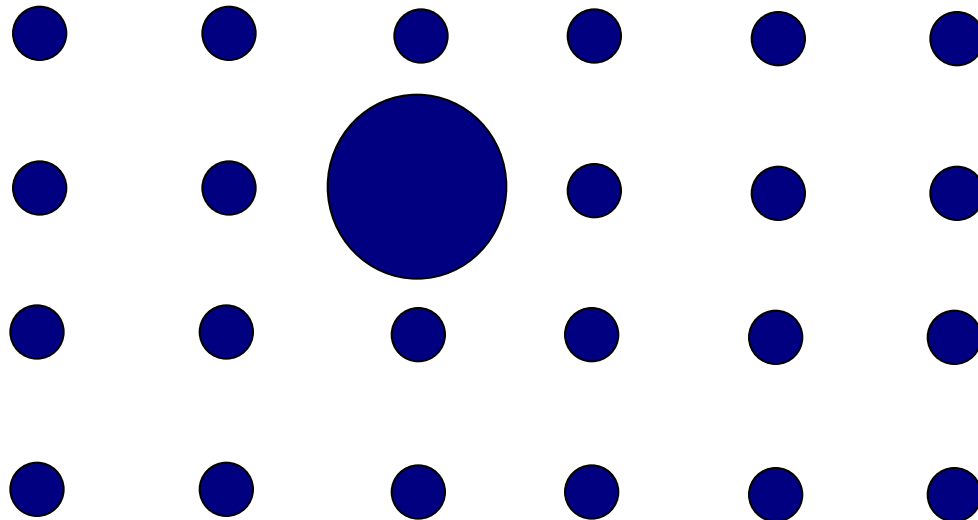
Stochastic models

- Possible states are given by possible value combinations for variables: $\mathbf{X} = \mathbf{V}$, with $\mathbf{V} = [V_1, \dots, V_n]'$
- Transitions between states are given by possible reactions k



Stochastic models

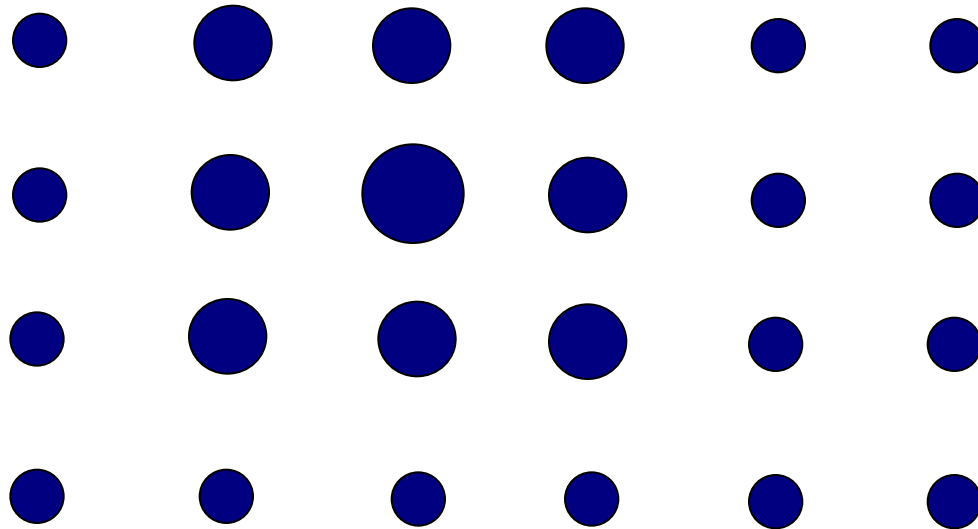
- Probability distribution $p[\mathbf{X}(t)=\mathbf{V}]$ describes probability that at time-point t there are $\mathbf{V} = [V_1, \dots, V_n]'$ molecules



Time t_0

Stochastic models

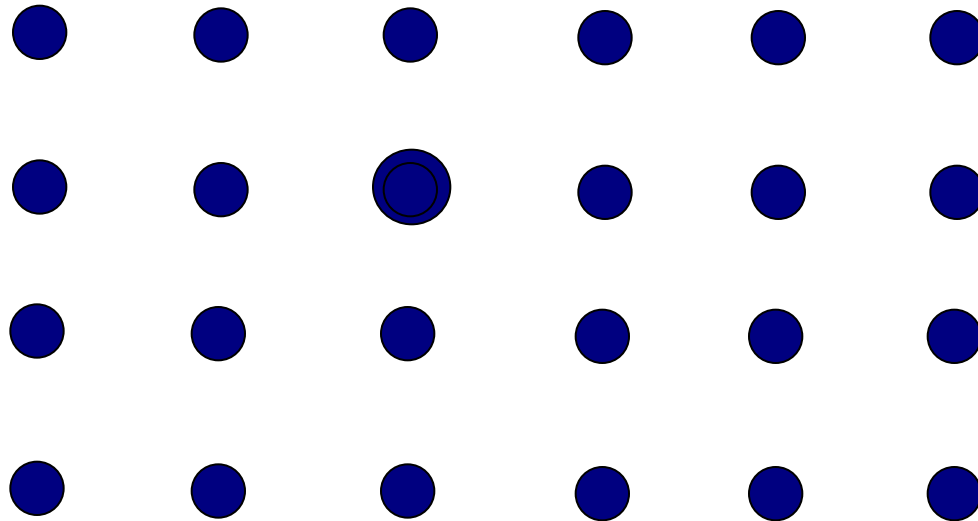
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Time t_1

Stochastic models

- Probability distribution $p[\mathbf{X}(t)=\mathbf{V}]$ describes probability that at time-point t there are $\mathbf{V} = [V_1, \dots, V_n]'$ molecules



Time t_2

Stochastic master equation

- Evolution of probability distribution $p[\mathbf{X}(t)=V]$ given by

$$p[\mathbf{X}(t + \Delta t) = V] = p[\mathbf{X}(t) = V] \left(1 - \sum_{j=1}^m \alpha_j \Delta t \right) + \sum_{k=1}^m p[\mathbf{X}(t) = V - \mathbf{v}_k] \beta_k \Delta t$$

- m is the number of reactions that can occur in the system
- $\alpha_j \Delta t$ is the probability that reaction j will occur in $[t, t + \Delta t]$ given that $\mathbf{X}(t) = V$
- $\beta_k \Delta t$ is the probability that reaction k will bring the system from $\mathbf{X}(t) = V - \mathbf{v}_k$ to $\mathbf{X}(t + \Delta t) = V$ in $[t, t + \Delta t]$

Van Kampen (1997), *Stochastic Processes in Physics and Chemistry*, Elsevier

Stochastic master equation

- For $\Delta t \rightarrow 0$ we obtain **stochastic master equation**

$$dp[X(t)=V] / dt = \sum_{j=1}^m p[X(t)=V-\nu_j] \beta_j - p[X(t)=V] \alpha_j$$

- Probabilities α_j, β_j are defined in terms of kinetic constants of reactions and number of reactant molecules
- Unimolecular reaction j : $S_1 \rightarrow \text{product(s)}$

$$\alpha_j = k_j X_1 (X_1-1)/2$$

- Bimolecular reaction j : $S_1 + S_2 \rightarrow \text{product(s)}$

$$\alpha_j = k_j X_1 X_2 / \Omega \qquad \Omega : \text{cell volume}$$

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic master equation

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- Probabilities α_j, β_j are defined in terms of kinetic constants of reactions and number of reactant molecules
- Analytical solution of master equation is not possible in most situations of practical interest

Van Kampen (1997), *Stochastic Processes in Physics and Chemistry*, Elsevier

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** predicts sequences of reactions that change state of system, starting from initial state $X(0) = V_0$

- Stochastic simulation samples joint probability density function

$$p[\tau, j | X(t) = V]$$

τ = time until occurrence of next reaction

j = index of next reaction

- **Interpretation:** $p[\tau, j | X(t) = V]d\tau$ is probability, given $X(t) = V$, that next reaction will occur in $[t + \tau, t + \tau + d\tau]$ and is reaction j

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $\mathbf{X}(0) = \mathbf{V}_0$
 - Stochastic simulation samples joint probability density function
$$p[\tau, j | \mathbf{X}(t) = \mathbf{V}]$$

τ = time until occurrence of next reaction

j = index of next reaction
 - Probability density function defined in terms of α_j, β_k (reaction constants)

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $X(0) = V_0$
- Stochastic simulation based on sampling of $p[\tau, j | X(t) = V]$ generates sequences in exact accordance with stochastic master equations
- Repeating stochastic simulation many times (Monte-Carlo procedure) yields approximation of probability distribution $p(X(t) = V)$

Gillespie (2002), *J. Phys. Chem.*, 81(25): 2340-61

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

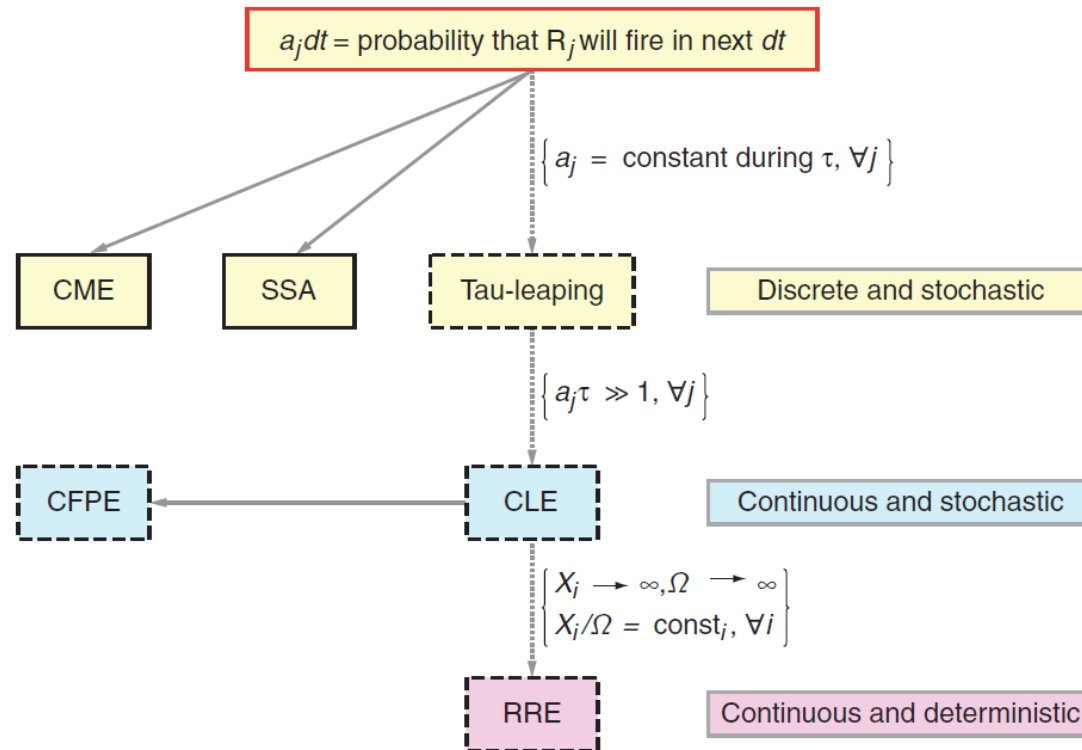
Stochastic simulation

- Analytical solution of master equations is not possible in most situations of practical interest
- **Stochastic simulation** generates sequences of reactions that change state of system, starting from initial state $X(0) = V_0$
- Various approximations of basic stochastic simulation algorithm, trading exactness for simulation speed:
 - Tau-leaping approaches: choose τ such that α_j, β_j remain approximately constant over time interval (encapsulate several reactions in one step)
 - Quasi-steady-state approximations (distinguish between slow and fast reactions)
 - ...

Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

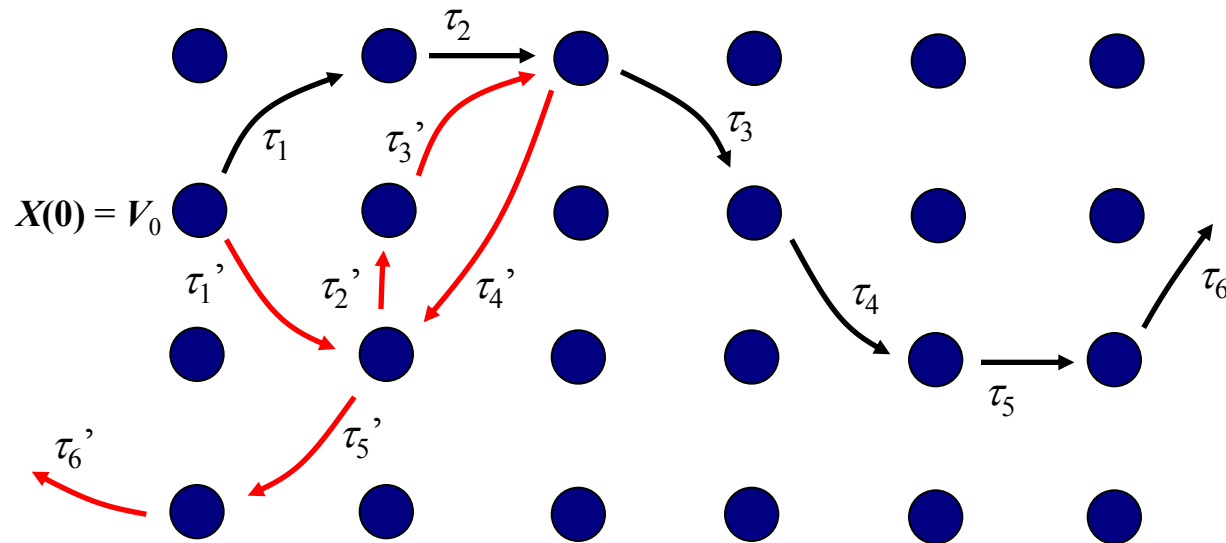
- Relation of stochastic simulation models with other modeling approaches



Gillespie (2007), *Annu. Rev. Phys. Chem.*, 58:35-55

Stochastic simulation

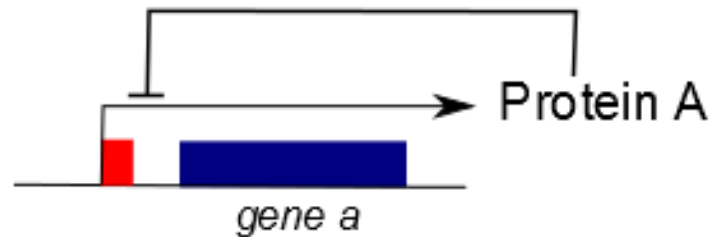
- Stochastic simulation generates sequences of reactions that change state of system, starting from initial state $X(0) = V_0$



- Stochastic simulation may lead to different dynamical behaviors starting from identical initial conditions: **heterogeneity**

Auto-inhibition network

- **Auto-inhibition** network consists of a single gene, coding for transcription regulator inhibiting expression of its own gene

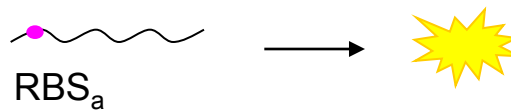
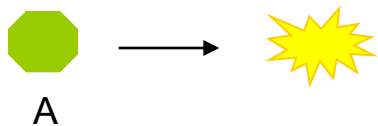
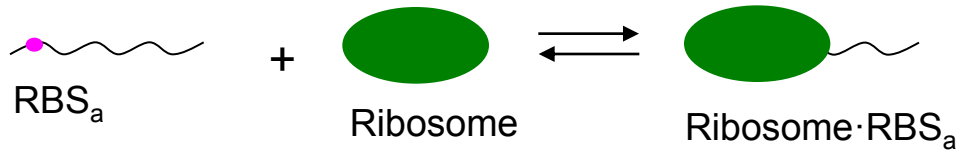
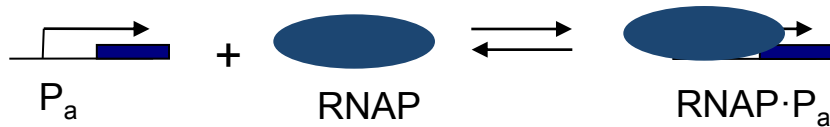


- Auto-inhibition is example of **negative feedback**, and frequently occurs in bacterial regulatory networks

Thieffry *et al.* (1998), *BioEssays*, 20(5):433-440

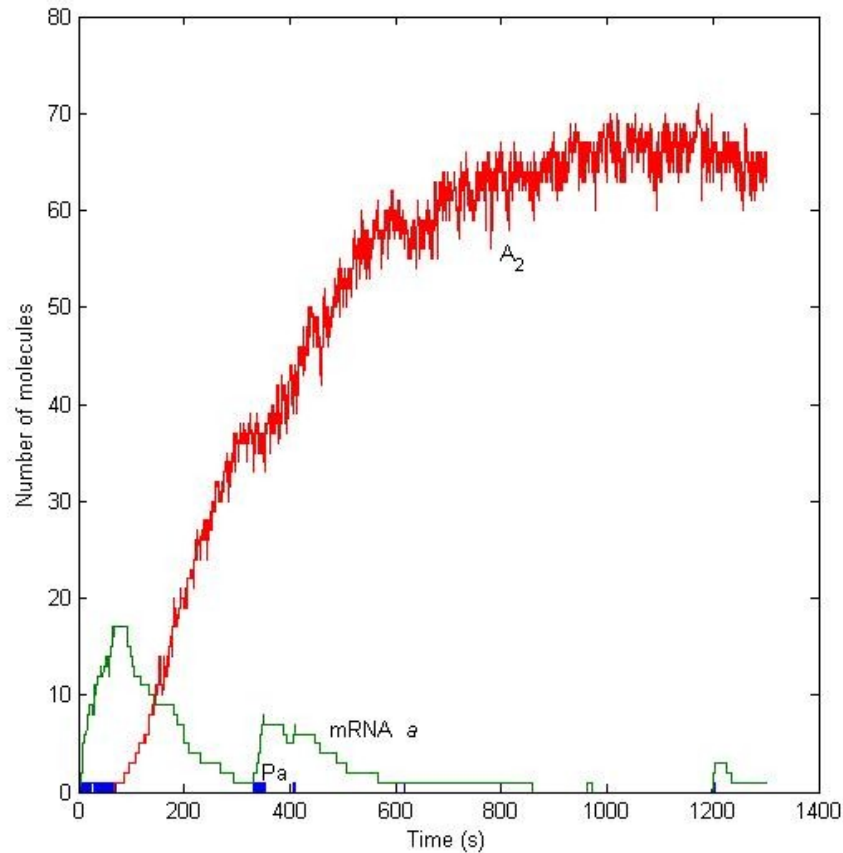
- Development of stochastic model requires list of species, reactions, and kinetic constants

Reactions and species



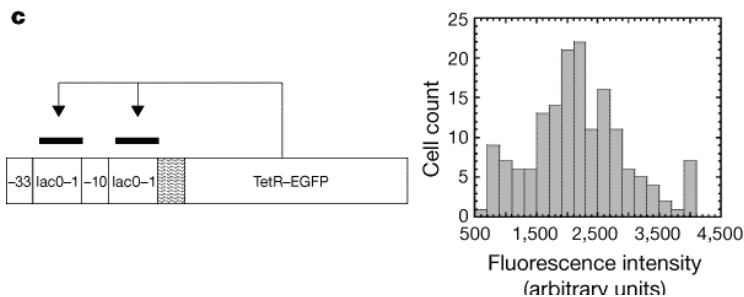
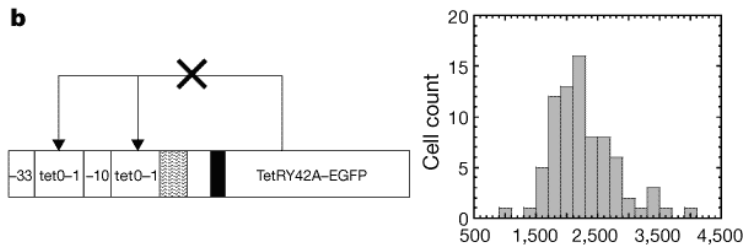
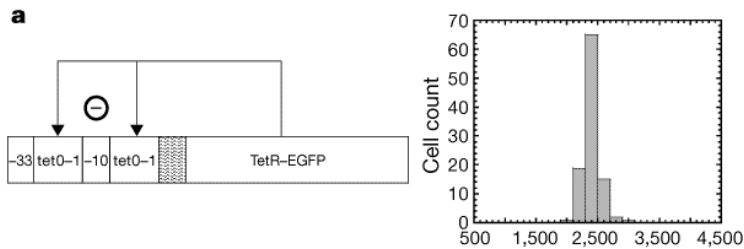
Stochastic simulation of auto-inhibition

- Occurrence of fluctuations and bursts in gene expression



Auto-inhibition and noise reduction

- Auto-inhibition reduces fluctuations in gene expression level

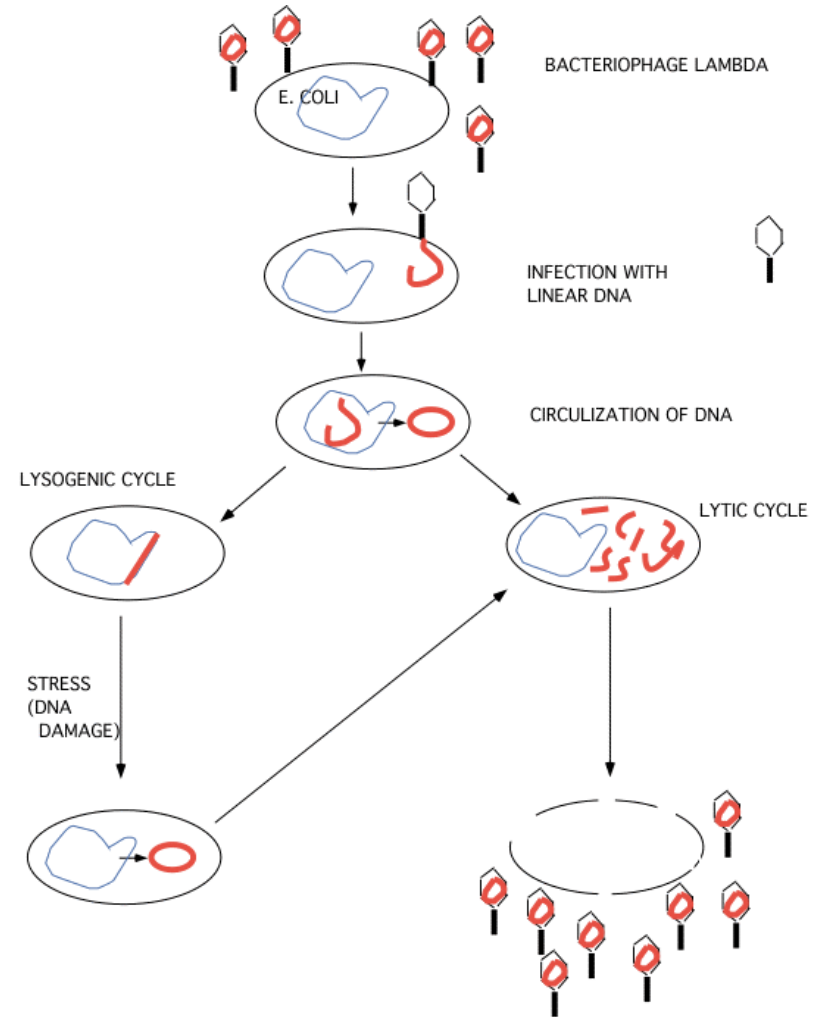
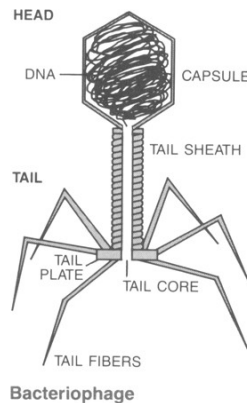


Becskei and Serrano (2000), *Nature*, 405(6785):590-591

Bacteriophage λ infection of *E. coli*

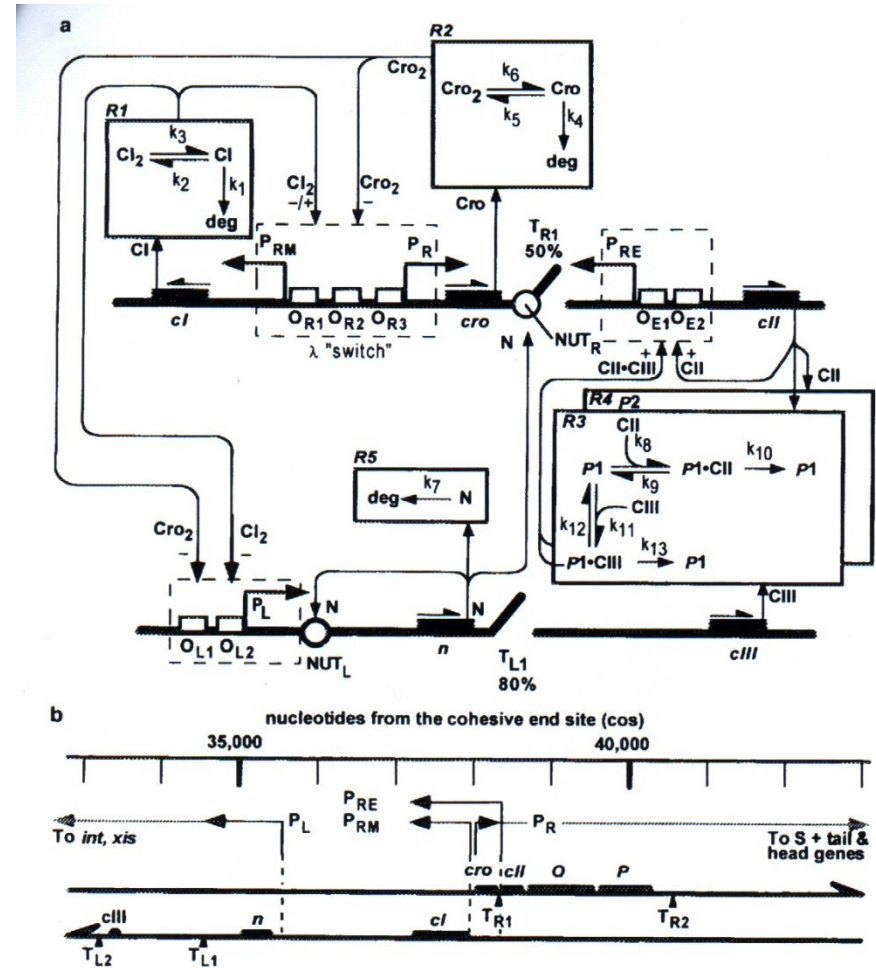
- Response of *E. coli* to phage λ infection involves decision between alternative developmental pathways: **lytic cycle** and **lysogeny**

Ptashne (1997), *A Genetic Switch: Phage λ and Higher Organisms*, Cell Press



Stochastic analysis of phage λ infection

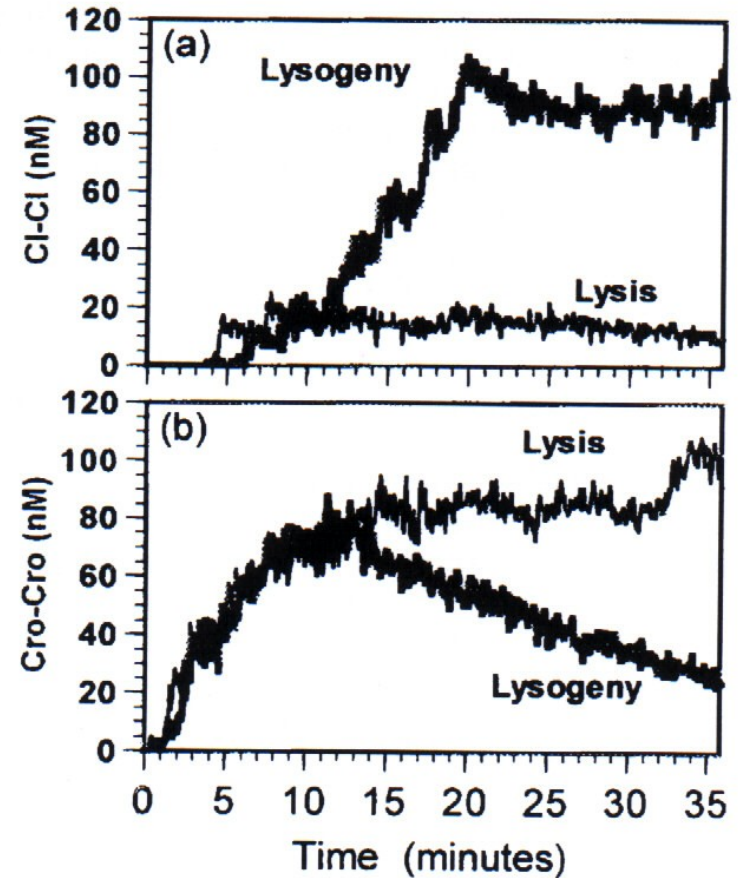
- Stochastic model of λ lysis-lysogeny decision network



Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

Stochastic analysis of phage λ infection

- Time evolution of Cro and CI dimer concentrations
- Due to stochastic fluctuations, under identical conditions cells follow one or other pathway (with some probability)



Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

Comparison with deterministic approach

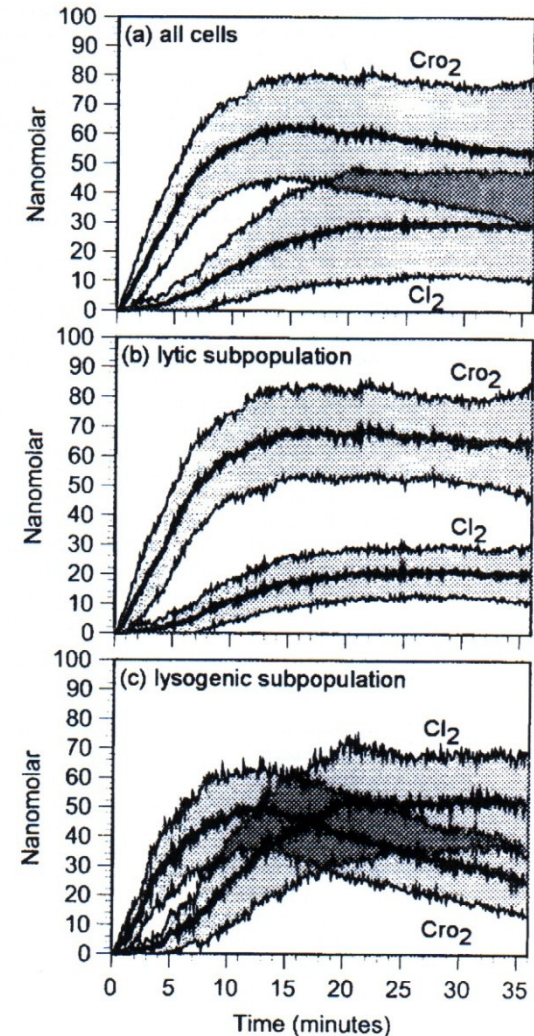
- Deterministic models can be seen as predicting **average behavior** of cell population

Gillespie. (2000), *J. Chem. Phys.*, 113(1): 297-306

- Analysis of average behavior may obscure that one part of population chooses one pathway rather than another

Arkin *et al.* (1998), *Genetics*, 149(4): 1633-1648

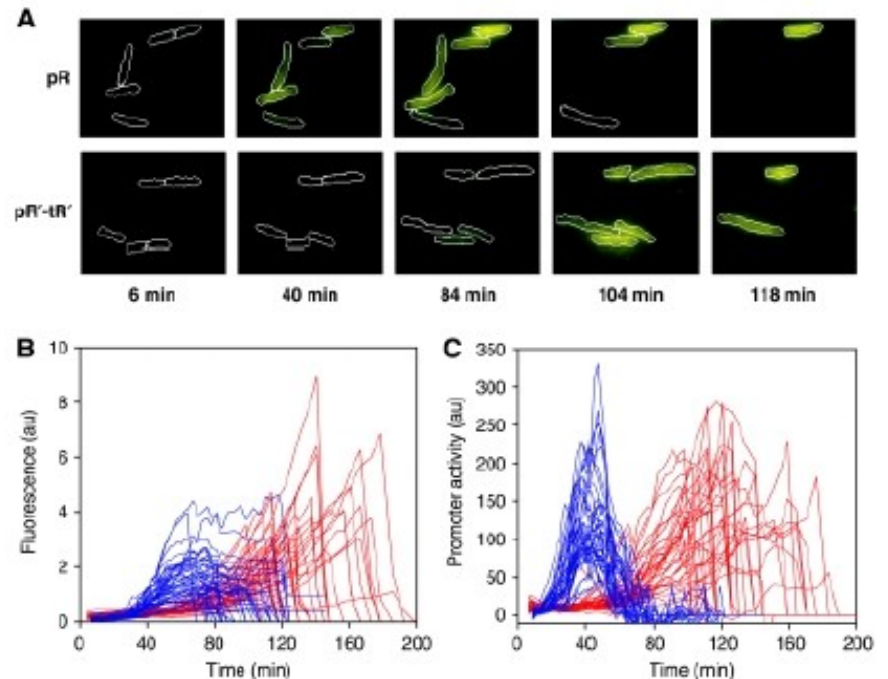
- However, under some conditions deterministic models yield good approximation



Measurements of phage λ infection

- New measurement techniques allow real-time and *in-vivo* monitoring of the execution of lytic and lysogenic pathways in individual cells

Use of reporter genes in combination with fluorescence microscopy

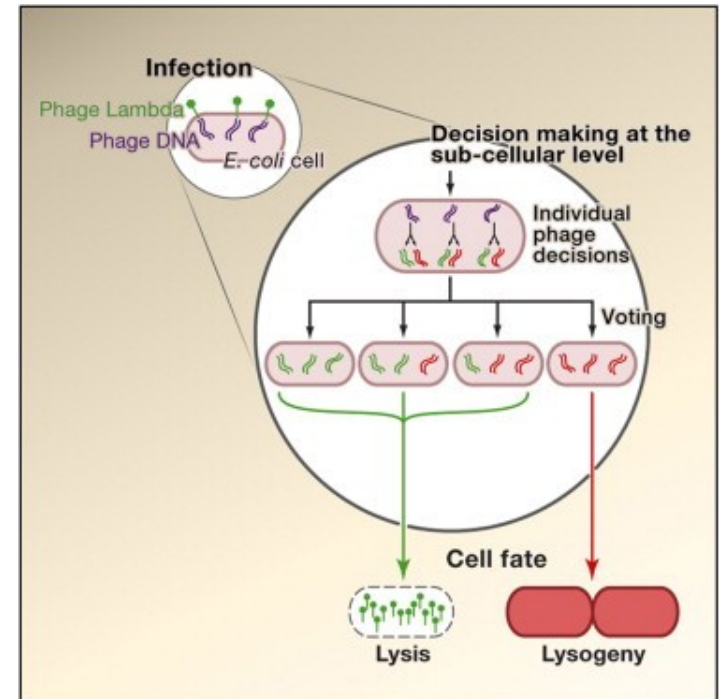
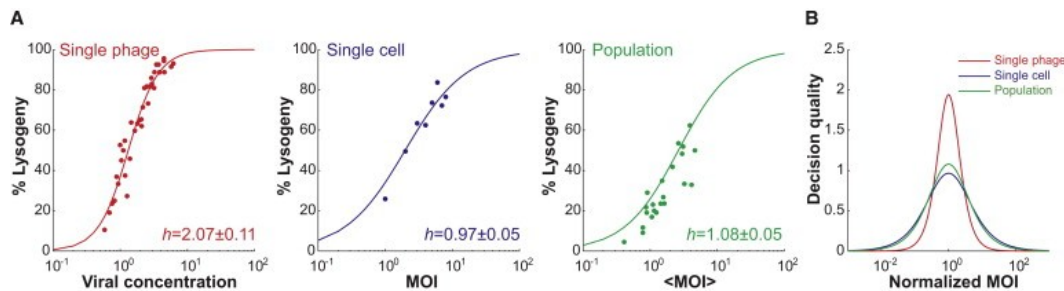


Amir *et al.* (2007), *Mol. Syst. Biol.*, 3:71

Stochasticity and hidden variables

- Is observed population heterogeneity entirely due to stochastic dynamics of biochemical reactions?
- **Hidden variables** that deterministically set outcome of what seems noisy decision process

Deterministic voting of stochastic decision in single phages



Zeng *et al.* (2010), *Cell*, 141(4):682-91

Conclusions

- Stochastic models provide more realistic picture of gene expression
- Difficulty of stochastic models is that required information on regulatory mechanisms on molecular level usually not available

Reaction schemas and kinetic constants, necessary for generating values of parameters τ and ρ , are not or incompletely known

- Another difficulty is that stochastic simulation is computationally expensive

Large networks cannot currently be handled, but a host of extensions and approximations have been developed

Merci !



www.inrialpes.fr/ibis